

CROP BREEDING, GENETICS & CYTOLOGY

Genetics of Winter Hardiness in 10 Lentil Recombinant Inbred Line Populations

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ABSTRACT

Available winter hardy lentil (*Lens culinaris* Medik.) germplasm has prompted interest in the development and use of cultivars that can be fall planted in cold highland areas. This change in production of lentil from normally spring sown to fall sown is environmentally sound and increases yield potential. Understanding the mode of inheritance of winter hardiness in lentil would assist breeding efforts. The objectives of this study were to determine the inheritance and heritability of winter hardiness in lentil. Ten F_6 derived recombinant inbred line (RIL) populations from crosses of winter hardy germplasm lines with nonhardy germplasm were planted in a randomized complete block design with three replications at Haymana, and Sivas, Turkey, and at Pullman, WA, USA, between 1997 and 2001. Meaningful data for an analysis of the inheritance of winter hardiness were available only at Haymana in 1997-1998 and at Pullman in 1998-1999, indicating infrequent occurrences of sufficiently cold winters to provide differential killing. Percent survival was calculated on the basis of plant stand counts recorded after establishment in the fall and regrowth in the spring. Parental line WA8649041 was the most winter hardy followed by WA8649090, ILL-1878, and ILL-669. General combining ability (GCA) and specific combining ability (SCA) effects were significant at both locations. Heritability estimates among the 10 RIL populations ranged from 15.9 to 90.7%. Inheritance patterns of winterhardiness appeared to be quantitative on the basis of frequency distributions and the lack of discrete segregation classes. Since winter hardiness in lentil appears to be a quantitative trait, accumulation of genes responsible for winter survival will probably require stringent field testing or marker assisted selection.

LENTIL is an annual self-pollinated diploid ($2x = 2n = 14$ chromosomes) species, and a highly valued food legume grown extensively in the Middle East, North Africa, North America, Australia, and South Asia. In the Palouse region of the U.S. Pacific Northwest, the crop is usually sown in early spring and harvested in late July or early August. However, winter hardy germplasm is available and may be used to develop higher yielding types that can be planted in the fall. With fall or early winter planting, lentil yields can be increased up to 50% (Sakar et al., 1988) by making more efficient

use of available moisture and by avoiding heat stress. Fall planting is desirable because drier soil conditions allow for planting the crop without the excessive soil compaction that is common with spring planting in cold wet soils. Spring planted lentil crops often experience heat stress and terminal drought in the latter part of the growing season that reduces yields. Crops planted in the fall may develop and mature sufficiently early to avoid the most severe heat and drought stresses.

In cold highland areas, winter lentils are not grown because cultivars with sufficient winter hardiness and acceptable quality traits are not available. Much of the work on lentil winter hardiness has been related to agronomic, physiologic, and germplasm screening (Kusmenoglu and Aydin, 1995; Eyupoglu et al., 1995; Summerfield et al., 1985; Erskine et al., 1981). On the basis of available germplasm, however, it should be possible to develop lentil cultivars with improved winter hardiness and acceptable quality (Erskine et al., 1981; Spaeth and Muehlbauer, 1991).

Genetic studies of winter hardiness in lentil are limited. A recent study (Ali and Johnson, 2000) indicated that winter hardiness had low to moderate heritability (32–71%). Genetic studies of winter hardiness in other food legumes have been investigated in more detail. Winter hardiness in pea (*Pisum sativum* L.) is controlled by dominant (Cousin et al., 1985) and additive genes (Auld et al., 1983) and by as many as three or four genes (Liesenfeld et al., 1986). Cold tolerance in chickpea (*Cicer arietinum* L.) is controlled by at least five genes with tolerance dominant over susceptibility (Malhotra and Singh, 1990).

Breeding for winter hardiness is considered a long-term objective because field tests required for differential killing of segregating material are unpredictable and infrequent, occurring perhaps only once every 8 to 10 yr (Lewitt, 1980). Winter hardiness is affected not only by tolerance to cold but by tolerance to factors such as frost heaving, water logging, freeze-thaw cycles, and diseases as well (Steponkus, 1978). Cultural practices including planting date, plant density, and depth of planting, also affect winter survival (Kusmenoglu and Aydin, 1995). However, among all these factors, cold temperature tolerance is the major component of winter hardiness. Selection for winter hardiness on the basis of artificial cold tolerance tests can be considered an indirect selection criterion. However, associations between winter hardiness and cold tolerance are not stable and vary among experiments, perhaps because of experimental design and artificial conditions (Gullord et al.,

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Abbreviations: GCA, general combining ability; $G \times E$, genotype \times environment; RIL, recombinant inbred line; SPC, specific combining ability.

Table 1. Characteristics of lentil parental lines used in the crosses.

Traits	Parental lines				
	ILL-669	ILL-1878	Precoz	WA8649041	WA8649090
Winter hardiness	Intermediate	Intermediate	Nonhardy	Hardy	Hardy
Plant height (cm)	10–20	12–22	11–21	9–23	21–23
Days to Flower (days)	53	50	48	68	58
Cotyledon color	Orange	Yellow	Yellow	Orange	Yellow
100 seed weight (g)	3.0	4.0	4.5	2.9	3.0
Seed coat color	Brown	Green	Green	Brown	Green

1975). Therefore, final evaluations for winter hardiness should be performed in the field (Murray et al., 1988).

Genetic studies of winter hardiness in various crops have been performed with segregating populations such as F_2 , F_3 , and backcross generations (Muehlbauer et al., 1971; Auld et al., 1983; Liesenfeld et al., 1986). These populations are quickly generated, and genetic variance (additive, nonadditive) and gene action (dominance vs. recessive), can be determined. However, replication of these segregating populations over time and years is limited, and they are not commonly being used in current quantitative trait mapping studies. Since winter hardiness studies require specific winter conditions that are infrequent, the use of recombinant inbred line populations (RILs) in studies of complex traits has advantages because of the ability to replicate over years and sites. Our objectives were to determine the inheritance and heritability of winter hardiness in promising lentil germplasm using recombinant inbred lines from crosses of winter hardy, intermediate winter hardy, and winter hardy lines in a half diallel design without reciprocals.

MATERIALS AND METHODS

Five lentil accessions were chosen as parents on the basis of their winter hardiness and contrasting morphological traits (Table 1). The four winter hardy germplasm lines (ILL-669, ILL-1878, WA8649041, and WA8649090) and one nonhardy parent ('Precoz = ILL-1405') were crossed in a half diallel mating design without reciprocals (10 crosses) in the greenhouse in 1992. F_2 populations from the 10 crosses were advanced by single seed descent to produce 1085 F_6 derived RILs (Table 2). The F_6 -derived RILs and parents were sown in the field in the spring of 1997 for seed increase.

Three experimental locations were chosen for winter hardiness field tests including Pullman, WA, USA (altitude 850 m); Haymana, Turkey (altitude 1050 m), and Sivas, Turkey (1285 m) and were representative of typical moderate to cold winter highland climates for midlatitude regions. Field evaluations were conducted at two locations (Pullman and Haymana)

Table 2. Population numbers, crosses and the number of recombinant inbred lines in each population.

Populations	Crosses†	Number of RILs
Pop1	Precoz/ILL-669	102
Pop2	ILL-669/ILL-1878	105
Pop3	WA8649090/ILL-669	104
Pop4	WA8649041/ILL-669	115
Pop5	Precoz/ILL-1878	105
Pop6	WA8649090/Precoz	106
Pop7	Precoz/WA8649041	101
Pop8	WA8649090/ILL-1878	108
Pop9	WA8649041/ILL-1878	118
Pop10	WA8649090/WA8649041	121

† First parent in the crosses was used as female and second parent was used as male.

in 1997–1998 and at three locations (Pullman, Haymana, and Sivas) in 1998–1999. Field evaluation experiments at Pullman were seeded in a conventionally tilled field on 15 Oct. 1997 and in minimally tilled fields with barley stubble on 5 Oct. 1998. At Haymana and Sivas locations, planting was performed in conventionally tilled fields. Planting dates for Haymana were 25 and 18 Oct. 1997 and 1998, respectively, and for Sivas was 25 Oct. 1998. Soil characteristics were fine silty, mixed mesic Pachic Ultic Haploxerollos at Pullman and silty-clay at Haymana. The 10 sets of RILs and parental lines were planted in a randomized complete block design with three replications. Plots were single rows 1 m long and spaced 0.3 m apart with an average of 30 to 40 plants in each row. A non-winter hardy check, 'Brewer', and a winter hardy check, WA8649090, were included after every 20 plots to compare with the RILs.

Winter survival of the RILs at each location was determined on the basis of plant stand counts recorded after complete emergence of seedlings in the fall and after regrowth in the spring. Daily air and soil temperatures were recorded hourly with data loggers from October to May at the Pullman location. Daily air and soil temperatures for Haymana location could not be recorded because of malfunction of the data loggers. Instead, monthly maximum, minimum, and average temperatures were obtained from Haymana Experiment Station, Turkey. The data loggers for recording air and soil temperatures were placed 250 mm above ground and 50 mm below ground, respectively.

Data from each recombinant inbred line population were analyzed separately by SAS software 6.12 (1996) PROC MIXED and PROC GLM models. Since the survival data were based on percentages, they were transformed by arcsine square root before analysis of variance. When no difference occurred between raw and transformed data, the raw data were used in statistical analyses. Adjusted least square means of RILs (i.e., average values of the genotypes adjusted for block effects) were used to determine the frequency distributions of the RILs for winter survival.

The heritability estimates for winter hardiness were calculated as the ratio of the genetic to the phenotypic variance from plot means (Fehr, 1987). Inheritance of winter hardiness was determined on the basis of frequency distributions for winter survival in each population. Since F_6 derived RILs are expected to be nearly homozygous, discrete segregation of 1 to 1 would be expected for single gene inheritance. A continuous distribution pattern for winter survival would indicate more than one gene conferred winter hardiness with probable environmental effects. General combining ability and specific combining abilities were calculated from mean survival of RIL populations on the basis of Griffing's half diallel analysis (Griffing, 1956). The program Dial 95 (Ukai, 1989) was used for diallel analysis.

RESULTS

Average monthly air and soil temperatures at Pullman from October to May 1997–1998 ranged from –1.7

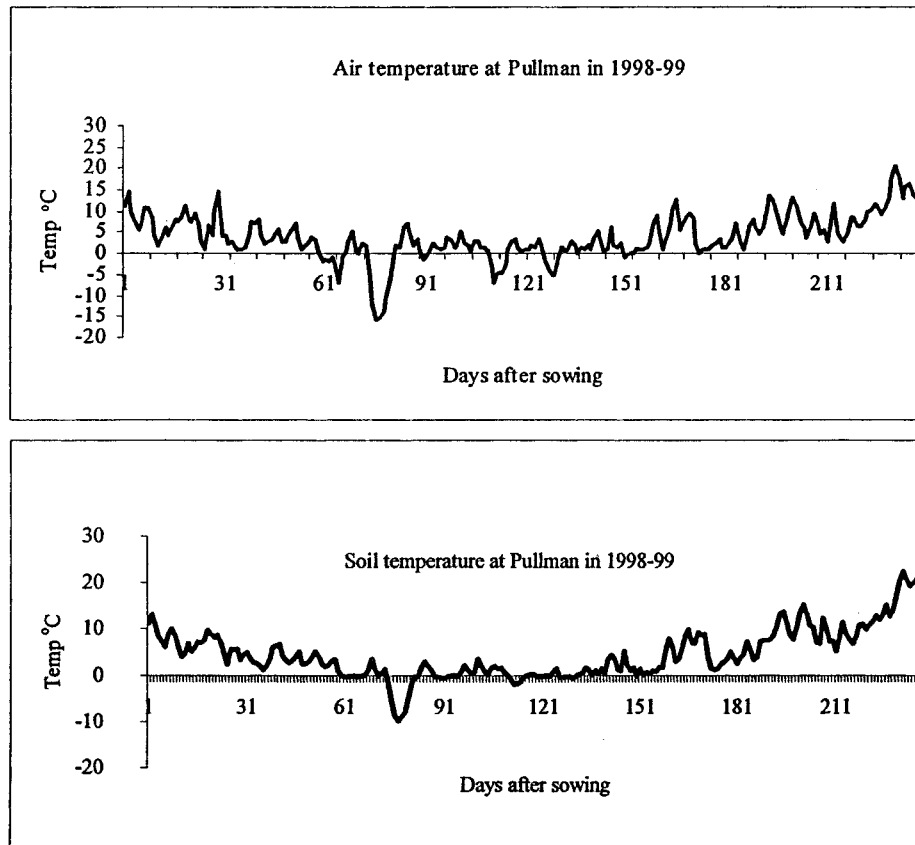


Fig. 1. Mean daily air and soil temperatures at Pullman, WA, 1998-1999.

to 10.8°C and from -0.9 to 13.1°C, respectively. Temperatures below zero were observed from October to May. The lowest average daily air and soil temperatures -19.5°C and -11.8°C, respectively, were recorded in December at Pullman in 1998-1999 (Fig. 1). At Haymana in 1997-1998, average monthly air temperatures from October to May ranged from -0.5 to 14.6°C and lowest air temperature recorded was -12.5°C. Temperatures below zero were observed from October to April. At Pullman, plants experienced low temperatures without snow cover while there was about 200 mm of snow cover in the winters at Haymana.

No winter killing was observed at Pullman in 1997-1998 while there was substantial winter killing during the winter of 1998-1999 (Table 3 and 4). At Haymana, there was moderate winter killing in the winter of 1997-1998 while no winter killing was present during the winter of 1998-1999. At Sivas, there was complete killing in the winter of 1998-1999.

Mean survival of the five parental lines ranged from 37 to 95% at Haymana in 1997-1998 and 0 to 76% at

Pullman in 1998-1999 (Table 3). Survival of the parental lines was different at both environments, but their survival rank did not change suggesting no genotype \times environment interactions ($G \times E$). The hardest parent was WA8649041, followed by WA8649090, ILL-1878, ILL-669, and Precos (Table 3). Percent survival of the hardest parent, WA8649041, was significantly greater than the other parental lines after the severe winter conditions at Pullman in 1998-1999.

Mean survival of the 10 RIL populations ranged from 47 to 86% at Haymana and 0 to 75% at Pullman (Table 4). Analysis of variance results were significant for all populations at both locations (Table 5). Survival at both locations was lowest for the nonhardy \times hardy crosses, while average survival was the highest for the hardy \times intermediate hardy crosses at Haymana and the hardy \times hardy cross at Pullman. One general observation from the Pullman field test was that as winter hardiness of the parents increased, mean survival of the RIL populations increased linearly in all crossing groups (Fig. 2). This general result suggested that the parental

Table 3. Mean winter survival (%) of parental lines at Haymana, Turkey, during the winter of 1997-1998 and at Pullman, WA, USA, during the winter of 1998-1999.

Parents	Winter hardiness	Haymana 1997-1998		Pullman 1998-1999	
		Mean	Range	Mean	Range
Precos	Nonhardy	37.0	25.0-54.2	0.0	0-0
ILL-669	Intermediate hardy	66.2	38.7-73.1	10.4	8.9-13.4
ILL-1878	Intermediate hardy	73.6	42.4-85.2	18.2	4.0-37.1
WA8649090	Hardy	77.5	61.5-86.3	26.0	17.0-33.5
WA8649041	Hardy	95.0	76.7-98.7	74.5	69.1-85.6

Table 4. The range, rank and mean winter survival (%) of recombinant inbred line populations at Haymana, Turkey, in 1997-1998 and at Pullman, WA, USA, in 1998-1999.

Pop	Crossing Groups†	Cross	Haymana 1997-1998			Pullman 1998-1999		
			Mean**	Range	Rank	Mean	Range	Rank
			Survival (%)					
Pop1	Nonhardy × intermediate hardy and hardy	Precoz/ILL-669	50.7 d‡	4.8–100.0	8	0.9 e	0–27.6	10
Pop5	Nonhardy × intermediate hardy and hardy	Precoz/ILL-1878	47.2 d	0.1–99.1	10	3.9 e	0–55.1	9
Pop6	Nonhardy × intermediate hardy and hardy	WA8649090/Precoz	49.6 d	7.4–93.6	9	5.2 e	0–62.1	8
Pop7	Nonhardy × intermediate hardy and hardy	Precoz/WA8649041	72.5 bc	12.4–105.7	3	27.6 c	0–96.4	4
		Mean	55.0			9.4		
Pop2	Intermediate hardy × intermediate hardy	ILL-669/ILL-1878	70.2 bc	12.3–100.0	6	10.8 de	0–88.5	7
Pop3	Intermediate hardy × hardy	WA8649090/ILL-669	65.5 bc	15.7–100.0	7	18.3 cd	0–96.0	6
Pop4	Intermediate hardy × hardy	WA8649041/ILL-669	70.3 bc	16.8–100.0	5	47.1 b	0–93.1	3
Pop8	Intermediate hardy × hardy	WA8649090/ILL-1878	71.9 bc	18.4–100.0	4	21.6 c	0–51.7	5
Pop9	Intermediate hardy × hardy	WA8649041/ILL-1878	86.0 a	7.7–100.0	1	63.9 a	0–100	1
		Mean	73.4			37.7		
Pop10	Hardy × hardy	WA8649090/WA8649041	72.6 b	19.5–100.0	2	55.7 a	0–92.5	2

† Nonhardy = Precoz, intermediate hardy = ILL-669 and ILL-1878, Hardy = WA8649041 and WA8649090.

‡ Means within locations followed by the same letter are not significantly different from each other as determined by a LSD at the 0.05 probability level.

Table 5. Analysis of variance results and heritability (h^2) estimates for winter survival at Haymana, Turkey, in 1997-1998 and at Pullman, WA, USA, in 1998-1999.

Population	Haymana 1997-1998				Pullman 1998-1999			
	MS	F	P	h^2 (%)	MS	F	P	h^2 (%)
Pop1	2026.4	4.59	0.0001	54.4	60.9	2.65	0.0001	35.4
Pop2	920.4	2.14	0.0001	27.5	431.2	4.66	0.0001	54.9
Pop3	945.2	3.32	0.0001	43.5	643.2	3.42	0.0001	44.6
Pop4	942.2	2.65	0.0001	35.4	1603.4	6.83	0.0001	73.3
Pop5	1620.6	4.75	0.0001	55.5	354.8	13.67	0.0001	60.5
Pop6	1774.3	4.74	0.0001	55.4	453.6	9.59	0.0001	74.1
Pop7	2181.8	6.27	0.0001	63.7	3021.5	30.36	0.0001	90.7
Pop8	998.8	1.57	0.0004	15.9	428.9	3.39	0.0001	44.3
Pop9	821.3	2.48	0.0001	32.9	1514.5	8.37	0.0001	71.0
Pop10	818.0	1.58	0.0002	16.2	1147.3	6.21	0.0001	58.8
Mean				40.1				58.8

lines had differing positive alleles that contributed to winter hardiness. At Haymana, the linear increase in mean survival of the populations was not obvious and might have been due to the relatively mild winter conditions and reduced winter killing of intermediate hardy lines.

Frequency distributions were continuous in most populations at Haymana, while at Pullman distributions were skewed toward the nonhardy parent in most populations (Fig. 2 and 3). In the presence of mild winter conditions at Haymana, frequency distributions were flat (e.g., Populations 1, 5, and 6) or skewed toward the mean of the winter hardy parent as in the case of Populations 8, 9, and 10. Under winter conditions at Pullman in 1998-1999, frequency distributions were mostly flat and continuous in crosses involving the hardest parent (Populations 4, 7, 9, and 10), while frequency distributions of other populations were skewed toward the mean of the nonhardy parent. No bimodal distributions for winter survival were observed among the populations. On the basis of frequency distributions, the results indicated that winter hardiness is probably controlled by several genes.

Winter conditions at Haymana in 1997-1998 were mild and it was difficult to differentiate among the lines or populations. However, in the presence of colder winter conditions at Pullman in 1998-1999, significant differences in survival were observed between the hardest and moderately hardy groups and among RILs within the populations. For example, at Haymana, mean sur-

vival of the hardy × hardy cross of Population 10 (WA8649041/WA8649090) was 72.6% and was similar to the survival of the hardy × nonhardy cross of Population 7 (WA8649041/Precoz), while at Pullman, mean survival of Population 7 (27.6%) was 50% lower than mean survival of Population 10 (55.7%) (Table 4).

Mean survival ranks of the 10 RIL populations at Haymana in 1997-1998 and at Pullman in 1998-1999 were similar with only minor shifts indicating no G×E interactions at the population level. For example, Populations 9 and 10 had the same survival ranking of at both locations, while Population 5 had the lowest mean survival and ranked 10 and 9 at Haymana and Pullman, respectively. However, significant differences were observed for the survival rank of individual RILs within populations at Haymana and Pullman locations suggesting significant G×E interactions.

Heritability estimates for the populations ranged from 15.9 to 63.7% at Haymana and from 35.5 to 90.7% at Pullman (Table 5). The heritability estimate was highest for Population 7 at both locations (63.7 and 90.7%, respectively), and similar heritabilities were estimated for Populations 2, 6, and 8 at both locations while heritability estimates were quite different for the other populations. The overall heritability estimate for winter survival at Haymana was 40.1% compared with 60.8% at Pullman (Table 5). When the difference between parents for winter hardiness was small, heritability estimates were low such as for Populations 2 and 10. This may indicate a lack of genetic variation in those crosses

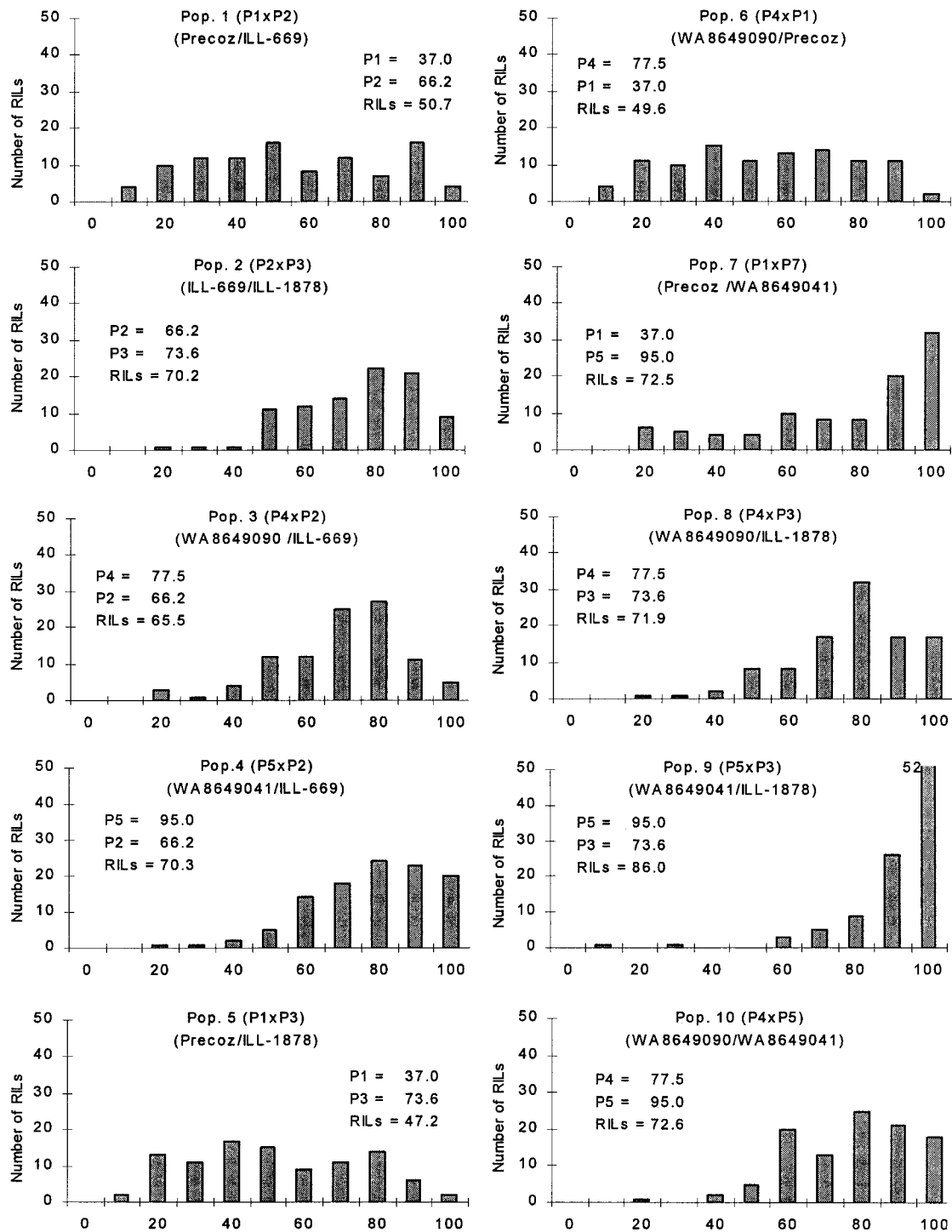


Fig. 2. Frequency distributions for winter survival of 10 RIL lentil populations at Haymana, Turkey.

and gains from selection may be small because of difficulties in differentiating individual lines for winter hardiness. Heritability estimates were high when parents differed widely in winter hardiness such as in Population 7, which was derived from a hardy \times nonhardy cross.

Estimated variances for GCA and SCA were significant at both locations (Tables 6, 7, 8). The mean square for GCA was greater than the mean square for SCA and indicated that GCA was a major source of variation for winter survival. Mean survival of the Precoz/WA-

8649041 cross combination with a SCA effect of 7.5% had better survival than expected at Haymana. Several crosses were significantly different from that expected on the basis of the GCA effects of the parents at Pullman, where winter killing was more severe. The cross ILL-1878/WA8649041 had high SCA effects, which accounted for an 8.2% increase in winter survival at Pullman. When survival of the RILs was evaluated for each cross, this same parental combination had the highest mean survival at both locations.

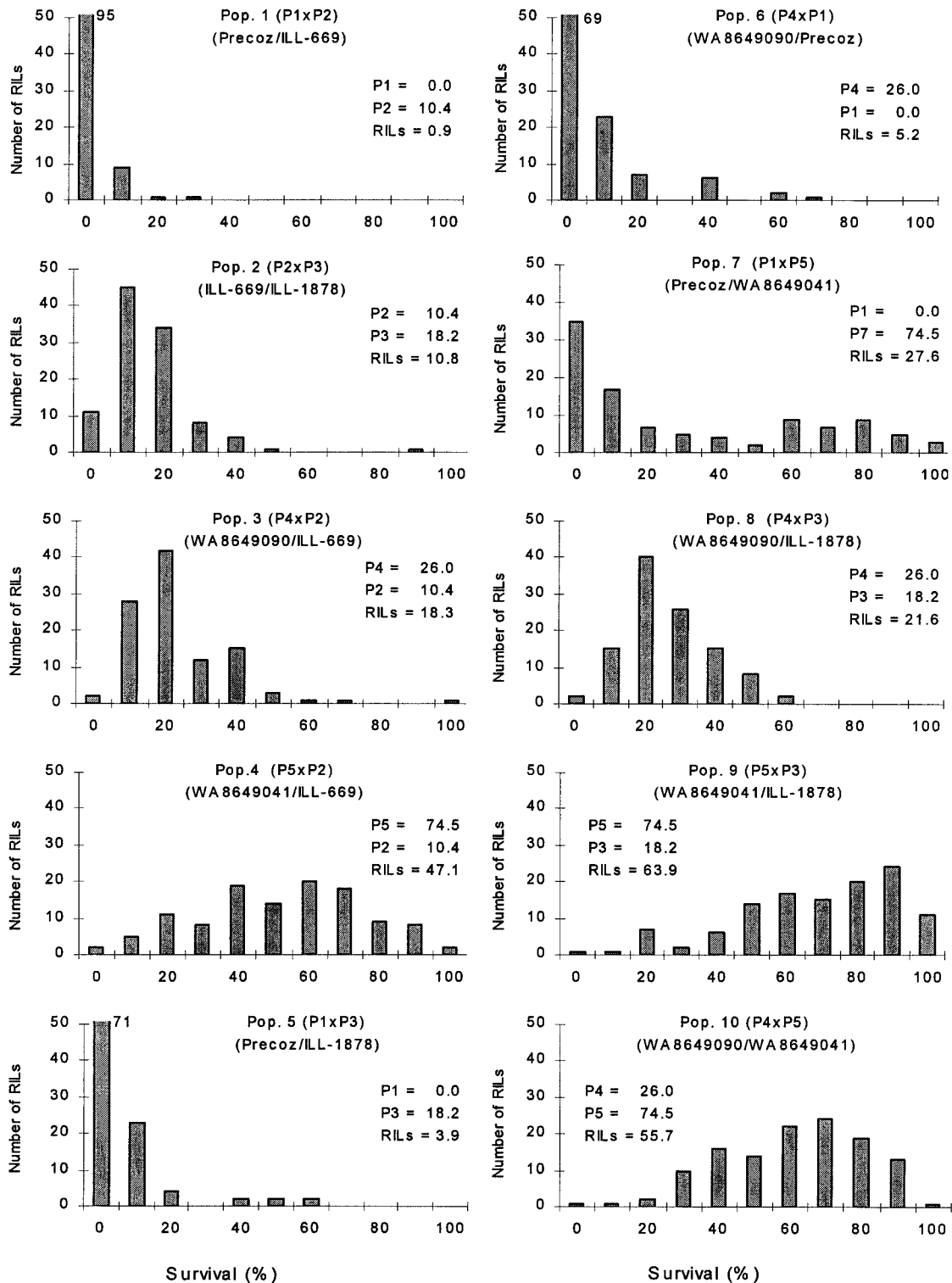


Fig. 3. Frequency distributions for winter survival of RIL populations from 10 crosses of lentil at Pullman, WA, USA, in 1998-1999.

DISCUSSION

Winter hardiness of 10 RIL populations of lentil appeared to be under polygenic control with additive loci because the mean of the progeny resembled the average of its parents (i.e., midparent value), while the opposite is true when a major gene is segregating in an F_2 population (Karlin et al., 1979). Frequency distributions for the 10 lentil populations were not consistent with a

single gene model for winter hardiness either as continuous frequency distributions are considered an indicator of polygenic inheritance (Lynch and Walsh, 1998). However, if environmental variation is large, segregation of major genes can be continuous (Lynch and Walsh, 1998). Frequency distributions for the 10 lentil populations for winter survival at two locations were not consistent with a single gene model for winter hardiness. Our

Table 6. General combining ability effects of the parental lines at Haymana, Turkey, in 1997-1998 and at Pullman, WA, USA, in 1998-1999.

Location	Precoz	ILL-669	ILL-1878	WA8649041	WA8649090
Haymana	-14.73	-1.14	3.67	13.78	-1.57
Pullman	-21.47	-8.27	-0.62	30.73	-0.37

Table 7. Specific combining ability effects of cross combinations at Haymana, Turkey, in 1997-1998 (first rows) and at Pullman, WA, USA, in 1998-1999 (second rows).

Parents	Precoz	ILL-669	ILL-1878	WA8649041	WA8649090
Precoz		0.51	-7.83	7.47	-0.15
		5.12	0.46	-7.17	1.58
ILL-669			1.56	-4.23	2.16
			-5.77	-0.84	1.48
ILL-1878				2.52	3.75
				8.19	-2.89
WA8649041					-5.77
					-0.18

results are in general agreement with reports by Thomashow (1990) on wheat (*Triticum aestivum* L.), Brouwer et al. (2000) on alfalfa (*Medicago sativa* L.), and Liesenfeld et al. (1986) on pea (*Pisum sativum* L.) that several genes control winter hardiness.

As the winter hardiness level of the parent increased, average survival of the populations increased as well (e.g., Populations 1, 4, 5, and 6). Liesenfeld et al. (1986) reported similar results in pea indicating that increasing dosage of the hardier parent increased the recovery of winter hardy lines from the segregating populations. Mean survival in crosses with the hardest parent (WA-8649041) was always higher than mean survival of the other crosses (Fig. 2); thus, to improve winter hardiness in development of future commercial winter hardy lentils, we suggest that WA8649041 should be used as the source of winter hardiness.

Although field tests are the ultimate measure of winter hardiness, major disadvantages include infrequent occurrence of winters with differential winter killing. In our case, at Pullman, there was differential winter killing in only 1 out of 5 yr of field tests. McIntyre et al. (1988) reported similar results for winter wheat indicating that differential winter killing occurred in only 1 out of 5 yr. We observed that, localized variations in soil temperatures, snow cover, water logging, and stubble distribution contributed to variable results. Experimental error associated with field tests is usually high which precludes detection of small differences in winter hardiness among progeny lines from hardy \times hardy crosses.

General combining ability effects were significant and largely higher than SCA effects indicating that selection for improved winter hardiness should be effective. Significance of GCA indicates that parents that combine well with a number of other parents may provide opportunities for breeding improved winter hardiness. The nonhardy parent Precoz had the largest negative GCA effects (-14.7 at Haymana and -21.5 at Pullman), while the hardest parent WA8649041 had the largest positive GCA effects (13.8 and at Haymana and 30.7 at Pullman) for winter survival indicating that this parent would be the best choice to include in crosses. ILL-1878 was also very good combiner for improved winter hardiness. Winter hardiness of Populations 7 and 9 showed good SCA and selection from Population 9 may produce lines with desired agronomic characters such as early flow-

ering and large seed size (Table 1). Similar results indicating the importance of GCA and SCA were reported in pea (Auld et al., 1983), oat (*Avena sativa* L., Jenkins, 1969; Muehlbauer et al., 1970), and wheat (Brule-Babel and Fowler, 1988; Sutka, 1984).

Heritability estimates ranged from low to high for winter hardiness among the 10 RIL populations. Similarly, low to high heritability estimates ranging from 30.0 to 84.4% for winter hardiness were reported in diallel crosses of wheat (Orlyuk, 1985). Also, our estimates of heritability generally agreed with previously published results for winter hardiness (Ali and Johnson, 2000), with the exception of Population 7. The high heritability estimate for this population (90%) could be due to high genetic variance resulting from a wide range for winter hardiness between the two parents, or biased selection during generation advance of lines (Ukai, personal communication). Bias in selection during the development of RIL populations is unlikely because all RILs were developed in greenhouse conditions with no exposure to cold. Genetic constitution of the parents and over wintering conditions can also greatly influence heritability estimates. For example, relatively mild winters or very harsh winters may not differentiate among the progeny and genotypic variability may be comparatively low with low heritability estimates. Therefore, heritability estimates cannot be generalized and should be interpreted with regard to specific environments under which it was obtained. Overall results suggest that selection for winter hardiness should be effective at Pullman where a more differential winterkill occurred and the average heritability estimate for the 10 populations was higher.

In conclusion, inheritance and heritability studies of

Table 8. Analysis of variance for general combining ability (GCA) and specific combining ability (SCA) for winter survival at Haymana, Turkey, in 1997-1998 and at Pullman, WA, USA, in 1998-1999.

Source	DF	Haymana		Pullman	
		MS	F	MS	F
Rep	2	0.06	NS	6.76	NS
GCA	4	954.30	84.24**	3316.95	200.80**
SCA	5	117.61	10.38**	115.22	6.95**
Error	18	11.33		16.50	

** Significant at $P < 0.01$.

NS = not significant.

winter hardiness under field conditions provided insight that winter survival is probably controlled by more than one gene and highly influenced by environment. To understand and characterize better the genetics of winter hardiness, we suggest that molecular markers be used to identify genomic regions involved in the expression of winter hardiness. Those markers may then be used in marker-assisted selection to minimize the need for field testing. Associations between winter hardiness and other plant traits also should be determined. Furthermore, cold tolerance studies under controlled environments should be performed to determine if selection for winter hardiness in lentil can be made on the basis of results of freezing tests. Freezing tests could be used to determine the importance of cold tolerance relative to other factors that determine winter survival.

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